PCR of CD28 → 440pos → Stop

Phenolpurification :

Synthesis . 304 nm

CD28/440 Primer: [illegible]
[illegible] Not I

CD28/Stop Primer: [illegible]
BamHI
XhoI

[illegible] Cleavage and Deprotection Kit Instructions

[illegible] Oligo 1000 after completion of the [illegible] to protect both you and the DNA

[illegible] AMA reagent [illegible] the supplied [illegible] sufficient for 50 nmole and 200 nmole syntheses. 1000 nmole [illegible] Important Note: The vials supplied with this kit contain [illegible] Common [illegible] materials such as EPR [illegible] are not acceptable and will [illegible] material into the AMA reagent. ✓

Attach the [illegible] syringe to the top of the synthesis column. Twist [illegible] to ensure a tight fit. ✓

Attach the vial containing the AMA reagent to the synthesis column [illegible] ✓

Invert the vial/column/syringe assembly so that the syringe is at the bottom and pump the syringe several times to make sure all air is displaced from the column. ✓

[illegible] the vial/column/syringe assembly so that AMA reagent remains within the column. Let sit at room temperature for 5 minutes. ✓

Position the vial/column/syringe assembly so that the vial is at the bottom. Pump the syringe several times to push all the AMA reagent into the vial.

Remove the vial.

Cap the vial tightly.

Place the vial in a heat block. A variety of heat/time regimens are acceptable. The following is a guide. (This is based on a heat block containing water at the listed temperature. If no water is present then add 5 minutes to the listed times to allow for the slower temperature equilibration in air.) ✓

[65° C 5 minutes]
55° C 10 minutes
[illegible]° C 30 minutes
[illegible]° C 90 minutes

To prevent sample blowout, cool the vial to room temperature or less before opening. (A [illegible] in ice water is sufficient.) ✓

Dry the sample to remove the AMA reagent before using. Drying by SpeedVac, [illegible] or with a stream of gas are all acceptable. Do not dry by heating alone.

→ Pump [illegible] 150
+ 750 [illegible] NH_3 [illegible]
[illegible]
~ 10 [illegible]
[illegible]
→ AC [illegible]
[illegible]
[illegible]
[illegible]

→ aliquoted [illegible]

b): 1:200 Dilution (2+ 4 µl)

	Sample	Wavelength	Reading		
	1	260.0nm	-0.3000 A		0.[illegible]
CD28 440 1:200	2	260.0nm	0.1749 A	→ 34.98	⇒ 0.[illegible]
CD28 STOP 1:200	3	260.0nm	0.1326 A	→ 36.52	⇒ 0.[illegible]
	4				

Read avg time: 0.50 Read Mode: [Abs]

total Amount:

gute Ausbeute !!! { CD28/440: 30nm ✓
CD28/STOP: 20nm ✓

PCR: in 50µl (Mastermix)

5µl Taq buffer 10x
1µl CD28 in Bluescript (3.5µg/µl)
4µl dNTP: (to 2,5 mM from CT) ⇒ 200µM
✓ 20 ← ~~30~~ µl H_2O
4µl $MgCl_2$ [25mM] → 2mM
a little bit to less ! ! 7µl $CD28_{440}$ [0.148mM] → 1:100: ~~1,48~~ 0.094 µM ⇒ ~~0.2~~ 0.15 µM
10µl $CD28_{STOP}$ [0.1mM] → 1:100: 1µM ⇒ 0.2µM.
0.5µl Taq [5U/µl].

Cycle file 19: 10' 95°C
1' 95°C }
1' 58°C } 40 cycles
3' 72°C extension }
→ 40 cycles

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1.5% Gel:

2) 5µl PCR → LO78 440

1) 4µl λ BstE I (2µg)

1

2

– 320bp

→ phenolisieren / Fällen

in 350µl 900µl

+ 35µl NaAc

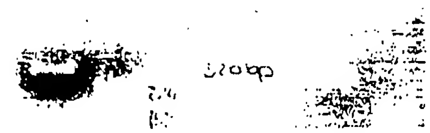
-20 °C

→ G 30'

→ 1 x w in 70% EtOH

→ 25µl H2O resusp. → 2µl on 1.5% Gel

6µl λ BstE I (3µg)



320bp

Not I Digest over night: in 30µl

20µl DNA → 3µl store for T-vector-Ligation!

3µl H2O

3µl NEB 3

3µl 10 x BSA

1µl Not I

17.2.96

20.2.96 BlnI-Digest O.N

30µl Not-Digest

✓ 1.5µl H2O → 21.2.96

✓ 0.5µl BSA 100x

✓ 3µl NEB 3

2µl BlnI

NEW Oligosynthese:

CD28 441:

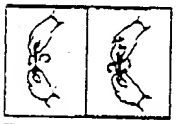
CD28/441 Primer: 5'-ataagtat *gcg gcc gca* att gaa gtt atg tat cct cct cc-3'
[=1µM/ml] |__ Not I__|

Tm=66°C

UltraFast Cleavage and Deprotection Kit Instructions

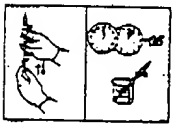
Remove the synthesis column from the Oligo 1000 after completion of the synthesis. You should wear gloves to protect both you and the DNA.

Use a pipettor to measure 0.5 to 1 ml of AMA reagent into one of the supplied vials. 0.5 ml is sufficient for 30 nmole and 200 nmole syntheses. 1000 nmole syntheses require 1 ml. Important Note: The vials supplied with this kit contain a special fluorocarbon O-ring. Common O-ring materials such as EPR, Viton, silicone, etc. are not acceptable and will leach material into the AMA reagent. ✓



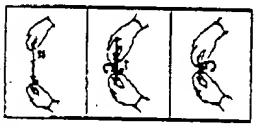
Attach the supplied syringe to the top of the synthesis column. Twist slightly to assure a tight fit. ✓

Attach the vial containing the AMA reagent to the synthesis column. Tighten firmly. ✓



Invert the vial/column/syringe assembly so that the syringe is at the bottom and pump the syringe several times to make sure all air is displaced from the column. ✓

Prop the vial/column/syringe assembly so that AMA reagent remains within the column. Let sit at room temperature for 5 minutes. ✓



Position the vial/column/syringe assembly so that the vial is at the bottom. Pump the syringe several times to push all the AMA reagent into the vial.

Remove the vial.

Cap the vial tightly.

Place the vial in a heat block. A variety of heat/time regimes are acceptable. The following is a guide. (This is based on a heat block containing water at the listed temperatures. If no water is present then add 5 minutes to the listed times to allow for the slower temperature equilibration in air.)

65° C	5 minutes
55° C	10 minutes
37° C	30 minutes
25° C	90 minutes

} tiefgefroren!!!

To prevent sample blowout, cool the vial to room temperature or less before opening. (A brief exposure to ice water is sufficient.)

Dry the sample to remove the AMA reagent before using. Drying by SpeedVac, lyophilization, or with a stream of gas are all acceptable. Do not dry by heating alone.

→ Resusp. in 200µl H2O
✓ + 2µl 1M → MgCl2/MgSO4 → 10mM f.c.
✓ 1ml EtOH
→ 40' -80°
→ 20' @ 4°C
→ Dissolve in
✓ 200µl H2O
→ aliquoted in 5 tubes → 15'

OD: 1:200 dilution (2µl add 400µl):

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CD28440 PCR-fragment (NotI / BNI Digested)
→ elution out of the gel for cloning:
1,2% LM-Gel

2x 80 µl CD28440 (NotI / BNI)
6µl Marker (3µg)
5µl PCR-CD28441 ✓ ~200ng
5µl negative control w/o template ✓

↓
cut off out!

⇒ scFv-STG (NotI/BNI) → preparative Gel from 16.2.96 (0.8%)
↓
Gel from 17/2/96

Cut out → melting in 65°C

↓
spin column